A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

NeoBiota 105: 47–65 (2026)
DOI: 10.3897/neobiota.105.165389

Research Article

Stage-based biotic resistance to a non-native species

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Abstract

Biotic resistance theory posits a negative relationship between diversity and invasibility. The mechanisms behind this can be variable, but studies are often focused on competition between many resident and introduced species. However, individual species may interact more strongly in communities, some of which may be effective predators and, thus, could offer stronger biotic resistance, broadening biotic resistance theory beyond diversity. In species with complex life histories, biotic resistance may be stage-specific, with resisting species affecting select life stages. The relationship between native eastern mosquitofish (*Gambusia holbrooki*) and the non-native tropical clawed frog (*Xenopus tropicalis*; TCF), provides a model system to investigate stage-specific biotic resistance. Preliminary observations suggest TCFs in their introduced range in Florida, USA are more likely to occupy ponds without fish and we hypothesise that mosquitofish provide predatory biotic resistance to early TCF life stages. We tested the hypothesis that the strength of predatory biotic resistance to species with complex life histories would be based on life stage. We also examined how the strength of predatory biotic resistance can be mitigated by predator density, cannibalism by adult life stages and habitat complexity. Hypotheses were tested using wild-caught and lab-reared individuals in controlled laboratory experiments. Early TCF life stages, lacking defensive traits, suffer near-total mortality due to predation and harassment, with vulnerability decreasing during and after metamorphosis. Mosquitofish density and habitat complexity had minimal effects, while the combination of adult frogs and mosquitofish resulted in facilitation between the two predators, increasing tadpole predation. This study underscores the need for thorough testing of the biotic resistance hypothesis across life stages and shows promise for the control of TCFs in Florida. Mosquitofish could be used as a biological control to reduce the recruitment of TCFs by eliminating the larval stage.

Key words: Amphibians, *Gambusia holbrooki*, predation, *Xenopus tropicalis*

Academic editor: Paul Teesalu
Received: 30 July 2025
Accepted: 10 December 2025
Published: 7 January 2026

Citation: Everett KM, Hill JE, Tuckett QM (2026) Stage-based biotic resistance to a non-native species. NeoBiota 105: 47–65. https://doi.org/10.3897/neobiota.105.165389

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Introduction

Elton's biotic resistance hypothesis is defined as a negative relationship between biodiversity and invasibility (Elton 1958; Levine and D'Antonio 1999; Beaury et al. 2020); the main mechanism is often considered to be interspecific competition between resident and introduced species (Levine et al. 2004). While the diversity-invasibility relationship has mixed support (Jeschke et al. 2012; Jeschke 2014), which is partly due to scale dependence (Davies et al. 2007), studies on specific species and interactions have greater support, such as those on strongly interacting species (Henriksson et al. 2015). Strongly interacting species can be competitors or predators (Britton 2012; Leclerc et al. 2020) and predation is a significant

structuring force in aquatic systems, including providing resistance to non-native species (Northcote 1988; Alofs and Jackson 2014; Hammerschlag et al. 2019). In aquatic ecosystems, predation affects the density and size structure of prey species due to differential susceptibility (Blumenshine et al. 2000). This is particularly pronounced in species with complex life histories, including those exhibiting a larval stage (Werner and Gilliam 1984). While some larval animals have anti-predator defences, such as spines or toxins, many are highly vulnerable to predation until they grow larger than what their predators can consume (Paradis et al. 1996; Benard 2004). For the invasion of species with complex life histories, interactions between an invader and predatory resident species may thus differ amongst life stages, with some vulnerable life stages experiencing stronger biotic resistance than others, potentially affecting invasion success or ultimate distribution; however, many studies of biotic resistance do not consider all life stages of the organism (Rius et al. 2014).

Differences in prey vulnerability amongst life stages can influence the strength of biotic resistance, particularly if predatory biotic resistance affects recruitment. Additionally, the interaction between biotic resistance and invader life history traits can also be modified by predator populations and communities, as well as ecosystem features (Jeschke et al. 2012). For variation in populations, predator density can alter the strength of biotic resistance (Twardochleb et al. 2012); greater densities of eastern mosquitofish (*Gambusia holbrooki* Girard, 1859) decrease survival of non-native poeciliid fishes in Florida (Thompson et al. 2012). For cannibalistic invading species with complex life histories and clearly vulnerable egg or larval stages, conspecific predation may play a significant role in the persistence of that species (Tinsley and McCoid 1996; Javidpour et al. 2020). This, in combination with interactions with resident predators, could result in variable outcomes of biotic resistance and invader establishment (Harvey et al. 2004; Skein et al. 2020). Finally, ecosystem variation can affect biotic resistance, including habitat complexity; for example, increasing structural complexity of the habitat can lower encounter rates between fish predators and prey, offering prey refuge and increasing survival (Osenberg and Mittelbach 1989). Investigating factors that limit or enhance biotic resistance will assist in explaining the context dependence of invasions.

The relationship between the native eastern mosquitofish and a novel amphibian invader, the tropical clawed frog (*Xenopus tropicalis* Gray, 1864), provides a model system for testing predatory biotic resistance and mitigating factors for a species with a complex life history. Tropical clawed frogs (hereafter TCF), an ephemeral pond-breeding amphibian from west-central Africa, were discovered to be reproducing in a suburban wetland in Florida, USA in 2016, the first such invasion globally (Hill et al. 2017; Goodman et al. 2021). Though the TCF has not been documented outside its native range prior to its recent invasion, a congener, the African clawed frog (*X. laevis* Daudin, 1802), is a global invader, with populations on four continents (Measey et al. 2012; Wang et al. 2019). Introduced populations of the African clawed frog have been implicated in the spread of chytrid fungus *Batrachochytrium dendrobatidis* (Soto-Azat et al. 2016), declines in native frog species (Lillo et al. 2011; Courant et al. 2018) and consumption of protected fish species (Duvall et al. 2025).

After the initial TCF breeding pond was discovered, larval stages were reported from up to 16 ponds and adult frogs in many more (Everett 2024). These breeding ponds were exclusively ephemeral and largely fishless, suggesting potential vulnerability of the larval stage to predatory fish (Goodman 2020). Observations made from the invasion of the African clawed frog in Chile suggest mosquitofish may

limit clawed frog invasion (Lobos 2020), which could be due to mosquitofish aggression (Baber and Babbitt 2003; Tuckett et al. 2021), although the mechanism was not fully investigated (Lobos 2020). Mosquitofish are native to the south-eastern USA, though they have established many invasive populations across the globe (Pyke 2008). Their primary impacts, both in their native and non-native range, are through predation and aggressive competition, consuming the eggs and biting the tail and fins of other aquatic animals (Komak and Crossland 2000; Pyke 2008; Tuckett et al. 2021). While these characteristics are problematic in their invasive ranges, mosquitofish are thought to be an important contributor to biotic resistance in their native range (Tuckett et al. 2017, 2021). Thus, it is possible that they could reduce the survival of TCF eggs and tadpoles if they were to occupy the same ephemeral habitats.

We focused on investigating stage-based prey vulnerability to mosquitofish predation and potential modifiers of biotic resistance to TCF invasion. We tested two hypotheses regarding the vulnerability of the frog to mosquitofish across its life stages: 1) biotic resistance would be variable across life stages and decreasing throughout the life of the frog and 2) this effect would be due to the changing morphology of the frog throughout its life, with mosquitofish attacking tadpoles' tails. We also tested three additional hypotheses that may explain changes in the interactions between TCF tadpoles and mosquitofish: 1) greater mosquitofish density may reduce TCF tadpole survival; 2) the presence of potentially cannibalistic adult TCFs may further reduce tadpole survival and 3) increased habitat complexity may offer tadpoles refuge from mosquitofish, increasing tadpole survival. We tested these hypotheses using wild-caught and captively spawned TCFs in a series of mesocosms and aquaria trials.

Materials and methods

Animal acquisition and husbandry

TCF adults and tadpoles were acquired from the wild population in Riverview, Florida and only used once in experiments. Frogs were captured in minnow traps baited with Purina Aquamax® Sport Fish pellets (Purina, St. Louis, MO), transferred to plastic bags, placed into an insulated tank for transport back to the lab and held for several days prior to the start of experimentation to ensure feeding. Some experiments were conducted outside of TCF breeding season; therefore, we used induced spawning techniques on the captive stock to produce the tadpoles used in experiments. Spawning procedures were adapted from Kashiwagi and Kashiwagi (2015). We first injected male and female frogs with a priming dose of 0.1 mL of human chorionic gonadotropin (Chorulon®, Merck Animal Heath, Rahway, NJ). We administered a second (resolving) dose of 1 mL 24 hours later and the mating pairs were placed into a large, insulated tank with degassed well water. Amplexus typically began within four hours, eggs were laid and the pairs were removed the following morning. The eggs were allowed to develop in the tank with daily water changes, until the tadpoles reached the free-swimming stage approximately four days later (Stage 45, Zahn et al. (2022)). Tadpoles were then placed into 38-litre aquaria with a flow of 25 °C well water. Hatchling tadpoles were fed spirulina (HR Supplements, Phoenix, AZ) twice daily; older tadpoles were fed a mixture of spirulina and powdered egg yolk once daily. Adult and juvenile frogs were housed in 38-litre aquaria and fed frozen bloodworms and Purina® Aquamax Starter pellets every other day in all trials. Uneaten food, waste and algae were regularly removed from the tanks by siphon.

Mosquitofish were raised in two onsite outdoor ponds (91 m^2 and 149 m^2) at the UF TAL and captured prior to trials with baited minnow traps. Fish used in trials where they were not able to be observed or replaced at death were dipped in a 32 ppt salt bath prior to stocking in experiments; this was done to remove external parasites and potentially enhance survival. Mosquitofish were fed Zeigler® Tropical Fish Flake (Zeigler Bros. Inc., Gardners, PA) once daily in all trials.

Life stage vulnerability

We tested mosquitofish predation on multiple TCF life stages: eggs, hatchlings (9 days post-fertilisation, avg. total length: 12.4 mm, SD = 1.50), small tadpoles (avg. TL: 18.3 mm, SD = 3.81), large tadpoles (avg. TL: 38.1 mm, SD = 4.82), metamorphs (forelimbs visible, avg. snout-vent length = 15.96 mm, SD = 1.90), juveniles (fully metamorphosed, but not sexually mature, avg. SVL: 18.5 mm, SD = 4.69) and adults (avg. SVL: 48.85, SD = 5.95).

The egg predation experiment took place in 10 75-litre tanks equipped with a centralised temperature control system. We placed four mosquitofish (two males and two females) into half the tanks two days before the start of the experiment to ensure feeding. All tank temperatures were set to 22 °C (using a combination of chilled water and a tank heater) similarly to Khokha et al. (2002) to maintain optimal developmental rates. We spawned frogs 12 hours before the start of the experiment. Forty eggs were then counted into plastic containers to keep the eggs in a confined area, then placed into the tanks. All remaining eggs were removed and counted after one hour to avoid hatching during the experiment.

Tank sizes varied between TCF life stages to account for differing body sizes. The hatchling experiment was carried out in 10-litre aquaria. The experiments using small tadpoles, large tadpoles and juveniles were done in 38-litre aquaria. The adult frog experiments took place in 75-litre aquaria. Lids covered each tank to ensure that no fish or frogs could escape. For each experiment, opaque barriers separated each tank to prevent visual interactions between neighbouring animals. A piece of PVC pipe (4.45 cm × 4.45 cm), with 8 strips of 2.54 cm wide black plastic tied to it, was placed in each tank to provide structure and refuge from mosquitofish. Aeration and an inflow of 25 °C well water were supplied to each tank in every experiment. Well water total ammonia nitrogen (0.20 ppm), nitrites (0.07 ppm), pH (7.75), hardness (444.6 ppm) and alkalinity (153.9 ppm) were measured with a Hach® kit (Hach Company, Loveland, CO, USA). We monitored water quality for the duration of each experiment and large deviations from baseline water quality of the well water were corrected by increasing flow to the tanks for a short time. All animals were fed as described above for the duration of each experiment.

Procedures and sample sizes were based on Tuckett et al. (2021). Each experiment consisted of eight control tanks and eight treatment tanks stocked with four mosquitofish, two males and two females (see Table 1 for number of replicates for each life stage). Two fish of both sexes were used to control for variation in behaviour between the sexes and between individuals; male mosquitofish are more aggressive than females, possibly because of their mating behaviour (Meffe and Snelson 1989; Magellan and García-Berthou 2015). We used four TCF individuals for the hatchling, small and large tadpole experiments, two individuals for the juvenile experiment and one individual for the adult experiment. Hatchlings, tadpoles and juveniles were added to each tank and allowed to acclimatise for two

Table 1. Summary of methods for each experiment in this study. Replicates (n), experimental enclosure volume, TCF life stage used, TCF density (TCF/m^2), mosquitofish density (fish/m^2), time given for acclimatisation, duration of the experiment and artificial vegetation stem densities (stems/m^2) for each experiment. For the life stage and structure trials, TCF and fish densities were calculated, based on the number of animals introduced and the bottom area of the tank (m^2). Stem density was calculated similarly for these experiments.

Experiment	n	Tank	Life Stage	TCF Density	Fish Density	Acclimation	Duration	Stem Density
Life-stage vulnerability	5	75-litre aquaria	Egg	252	25	None	1 hour	50
	8	10-litre aquaria	Hatchling	67	67	2 days	1 week or death	133
	8	38-litre aquaria	Tadpole	31	31	2 days	1 week or death	62
	8	38-litre aquaria	Metamorph	31	31	Until NF stages 59–60	1 week or death	62
	8	38-litre aquaria	Juvenile	16	31	2 days	1 week or death	62
	8	75-litre aquaria	Adult	6	25	Until feeding	1 week or death	50
Stocking density	5	516-litre vat	Tadpole	19	0, 8, 19, 42	3 days	1 week	20
Cannibalism	5	516-litre vat	Tadpole and Adult	19, 2	19	3 days	2 weeks	20
Structural complexity	8	38-litre aquaria	Tadpole	31	31	2 days	1 week or death	0, 62, 123, 246

days to account for any possible handling mortality. Any TCF individuals that died during this acclimatisation period were replaced. The adult frogs were allowed to acclimatise until they resumed normal feeding behaviour (between 1-2 weeks) after being transferred into the 75-litre tank.

At the end of the acclimatisation period, we placed a barrier inside the tank and added the four mosquitofish. They were given 20 minutes to acclimatise before we removed the barrier and a 20-minute period of observation began. We recorded any chases or bites by the mosquitofish (Magellan and García-Berthou 2015). We observed every life stage, except the hatchlings, which were too small at the beginning of the experiment to reliably observe. After the observation period, the experiment ran for one week. Each tank was checked twice daily for mortalities. We removed the tadpoles upon death or at the end of the week and the presence or absence of tail damage was recorded (Fig. 1). Tadpoles that were not recovered were assumed to be eaten whole and were counted as having damaged tails. If a fish died during the experiment, we replaced it with another individual of the same sex to maintain mosquitofish density. Tadpoles and frogs were not replaced if they died. The small tadpole and juvenile experiments were each split into two time blocks due to tank and animal availability. Each block was split into an even number of controls and fish treatment replicates.

The metamorph experiment was conducted in a similar fashion to the small and large tadpole experiments. We placed four large tadpoles close to metamorphosis into each tank and allowed them to develop until they reached metamorphic climax, after the appearance of fore-limbs (NF Stages 59-60). The experiment was begun as described above and continued until all metamorphs in a tank had either died or after one week. Tail damage was not recorded because the frogs' tails are re-absorbed during metamorphosis. This experiment was also split into two time blocks, with four control and four fish treatment replicates.

Stocking density

We determined how mosquitofish density affects tadpole survival. We placed thirty tadpoles (density = 18 tadpoles/m^2) in concrete vats with a bottom area of 1.69 m^2 and a depth of 0.3 m. To provide cover, artificial vegetation made of 2.54-cm-wide black plastic strips attached to a plastic grid were placed in the vats

Figure 1. Example of mosquitofish-induced tailfin damage on tropical clawed frog tadpoles. These images include three life stages: hatchling (top), small tadpole (middle) and large tadpole (bottom). Size scales differ between life stages.

and weighed down with rocks or capped PVC pipes filled with sand (stem density: 20 stems/m^2). Vats were covered with lids to ensure that no fish or frogs could escape. We used three stocking densities: 5, 11 and 25 fish/m^2. These densities were based on the methods of Thompson et al. (2012) and are comparable to the densities of wild mosquitofish. Only female fish were stocked to control for differences in behaviour between sexes. Animals that died during the course of the experiment were not replaced. At the end of the experiment, all fish and tadpoles were removed and counted and surviving tadpoles were assessed for tail damage.

Cannibalism

We investigated how the presence of adult frogs affects the survival of tadpoles, both with and without the presence of mosquitofish. This experiment was conducted similarly to the stocking density experiment, using the same concrete vats and artificial vegetation. We stocked tadpoles in each vat at a density of

18 tadpoles/m^2 and they were there for three days of acclimatisation before stocking adult frogs or mosquitofish. Four treatments were included: a tadpole-only control, tadpoles and 19 mosquitofish (11 fish/m^2), tadpoles and three adult frogs (2 frogs/m^2) and a treatment including all three. Densities of fish, frogs, tadpoles and artificial stems approximated densities observed from field sampling conducted in 2021 and 2022 (Everett et al. 2023). We allowed tadpoles to acclimatise for three days before any fish or adult frogs were added and the experiment was allowed to run for two weeks. Only female fish were stocked in this experiment and any fish that died during the course of the experiment were not replaced. At the end of the experiment, all animals were removed and counted and any surviving tadpoles' tails were assessed for damage.

Structural complexity

We conducted another experiment to assess how structural complexity and stem density affects mosquitofish predation and tadpole survival. We placed pieces of PVC pipe with black plastic strips tied to it into 38 litre tanks at one of four stem densities: eight stems (62 stems/m^2), 16 stems (123 stems/m^2), 32 stems (246 stems/m^2) or no stems. Each set of eight stems was attached to one piece of 4.45 cm × 4.45 cm PVC pipe and the control had no PVC pipes. We added four tadpoles to each tank and allowed them to acclimatise for two days. We then added two male and two female mosquitofish and the experiment was allowed to run for one week. All animals were fed as discussed above and tanks were checked for mortalities twice daily; dead mosquitofish were replaced, but dead tadpoles were not. Dead tadpoles were removed and checked for tail damage and the date of their death was recorded.

Statistical analysis

The structure, stocking density and multiple life stage predation experiment were split into time blocks due to tank and animal availability, as well as some of the life stage vulnerability trials (small tadpole, metamorph and juvenile). Each time block contained an even number of controls and fish treatment replicates. Tropical clawed frog mortality from the life stage and structure experiments were non-normal and, therefore, we analysed trials that were not time blocked with Mann-Whitney U tests (Quinn and Keough 2002); time-blocked experiments were analysed with Friedman's test (Pereira et al. 2015). We analysed the number of individuals with tail damage from the small and large tadpole experiments using the same statistical tests. Very few interactions between mosquitofish, tadpoles or frogs were observed and, thus, no analysis was performed on the behavioural observation data. We analysed the proportion of tadpoles that died in the stocking density experiment using a generalised linear model with a beta distribution, with dependent variables stocking level and time block (Douma and Weedon 2019). Tadpole survival from the cannibalism experiment was over-dispersed and was analysed using a generalised linear model with a beta binomial distribution (McCullagh and Nelder 1989). Four variables were included in this model: time block, presence of adult frogs, presence of mosquitofish and an interaction between the presence of adults and mosquitofish. All analyses and models used a Type-I error rate of $\alpha = 0.05$. Data for each experiment can be found in the Suppl. material 1.

Results

Life stage vulnerability

Mosquitofish significantly reduced the survival of eggs and all larval frog life stages (Fig. 2). They consumed all but one TCF egg within one hour; conversely, only one egg was not located at the end of the experiment in the control tanks ($\chi^2_{1,7}$ = 2.67, P < 0.01). No hatchling tadpoles survived exposure to mosquitofish, while only one control died ($\chi^2_{1,13}$ = 3.71; P < 0.01). In the small tadpole experiment, 86% of the tadpoles exposed to mosquitofish died and no control tadpoles died ($Z_{1,13}$ = 2.41, P = 0.02). No analysis was performed for the large tadpole experiment, because 100% of the tadpoles exposed to mosquitofish died, whereas no tadpoles in the control died. Mosquitofish also caused significant mortality to metamorphs ($Z_{1,13}$ = 2.26, P = 0.02), but mortality decreased to 65%. No juvenile or adult TCFs died in either the control or mosquitofish tanks and, thus, no analysis was necessary for these experiments. Time block was not significant in the small tadpole or metamorph experiments ($\chi^2_{1,13}$ = 0.39, 0.34; P = 0.53, 0.56).

Tail damage was only present for tadpoles that were exposed to mosquitofish. No control tadpoles exhibited any damage. In the small tadpole experiment, five tadpoles survived the mosquitofish, four of which did not have any tail damage. Tadpoles exposed to mosquitofish showed significant amounts of damage ($Z_{1,13}$ = 2.43, P = 0.01), as opposed to tadpoles not exposed to mosquitofish. Time block had no detectable effect on this experiment ($\chi^2_{1,13}$ = 0.40; P = 0.53). No analysis was performed for the large tadpole experiment because 100% of the tadpoles exhibited tail damage. Chasing and biting behaviour were rarely observed, with a total of only

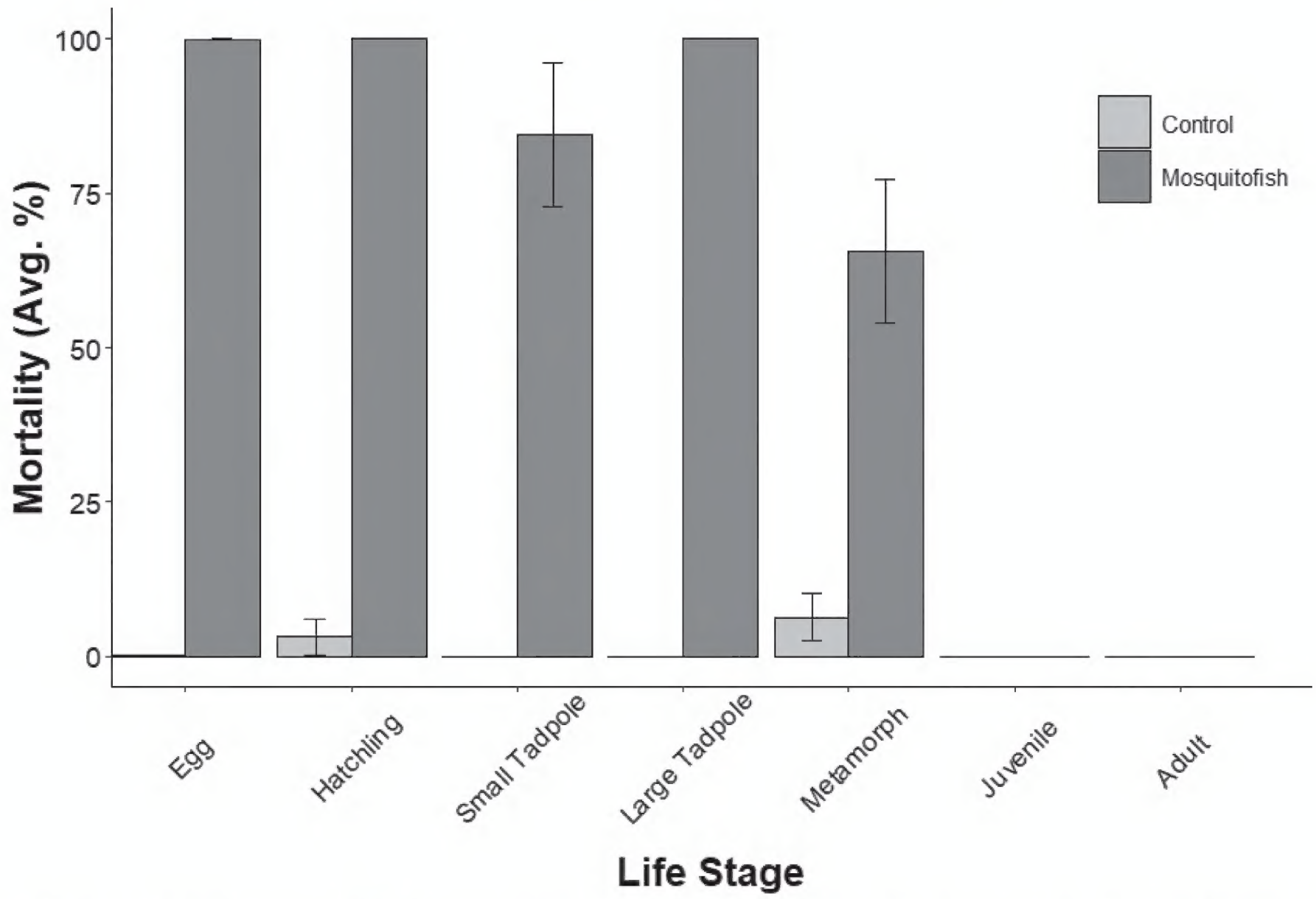

Figure 2. Average mortality (± SE) of TCF life stages in control and mosquitofish tanks. No mortality of controls was observed in the small and large tadpole trials and no mortality of juveniles or adults occurred in either the control or fish treatment tanks.

32 interactions between the mosquitofish and all frog life stages across 32 tanks. Eighteen of these were from a single tank in the small tadpole experiment. Based on the presence of the tail damage when the tadpoles were moved from the tank, it is likely that these types of behaviour occurred outside the window of observation.

Stocking density

The 5, 11 and 25 fish/m^2 stocking densities led to variable tadpole mortality ($\chi^2_{3,17} = 43.00$; $P < 0.01$). Time block had no effect on this experiment ($\chi^2_{4,17} = 3.52$; $P = 0.47$). The two highest densities, 11 and 25 fish/m^2, resulted in 99.7% tadpole mortality (Fig. 3A). The single surviving tadpole metamorphosed during the trial. The 5 fish/m^2 stocking density had lower mortality than the two higher densities of fish, but was not low enough to be significantly different (Fig. 3A). More than 12 times less tadpoles died when no fish were present (avg. tadpole mortality = 8%, SD = 6.86). All surviving tadpoles in the three mosquitofish treatments exhibited tail damage, whereas only six control tadpoles had damage (4%).

Cannibalism

The combination of the adult TCFs and the mosquitofish resulted in the highest tadpole mortality (average tadpole mortality = 96%, SD = 4.9). Tadpole mortality was highly variable in the mosquitofish and tadpole only treatment, ranging from 0 to 97% tadpole mortality (Fig. 3B). The adult TCF treatment resulted in very low tadpole mortality, approximately 32 times lower than the combination of adult TCFs and fish (average tadpole mortality = 3%, SD = 2.5), similar to the controls with tadpoles only. However, both adult TCFs and mosquitofish affected tadpole mortality, with adult TCFs only influencing tadpole mortality in the presence of the mosquitofish (Table 2). Of the tadpoles that survived, 99% of the tadpoles exposed to fish (mosquitofish and mosquitofish + adult TCF treatments) had damage to their tails, while < 1% of the tadpoles not exposed to fish (control and adult TCF treatment) had tail damage. The tadpoles that died during the experiment were not recovered and, thus, not scored. One replicate of this experiment was excluded after many of the fish were found to be heavily infected with nematodes (*Huffmanela* cf. *huffmani*). The replicate was re-run later with healthy fish.

Table 2. Results of the cannibalism experiment using a generalised linear model (beta binomial distribution). Treatments included a tadpole-only control, mosquitofish, adult TCFs and the combination of all three.

Parameter	DF	Wald χ^2	P
Fish	1	78.41	< 0.001
Adults	1	33.44	< 0.001
Fish*Adults	1	43.72	< 0.001
Time Block	4	16.03	< 0.001

Structural complexity

Only 2% of tadpoles survived to the conclusion of the experiment (Fig. 3C). Stem density had no effect on tadpole survival ($\chi^2_{3,29} = 0.20$; $P = 0.98$). All tadpoles exhibited tail damage.

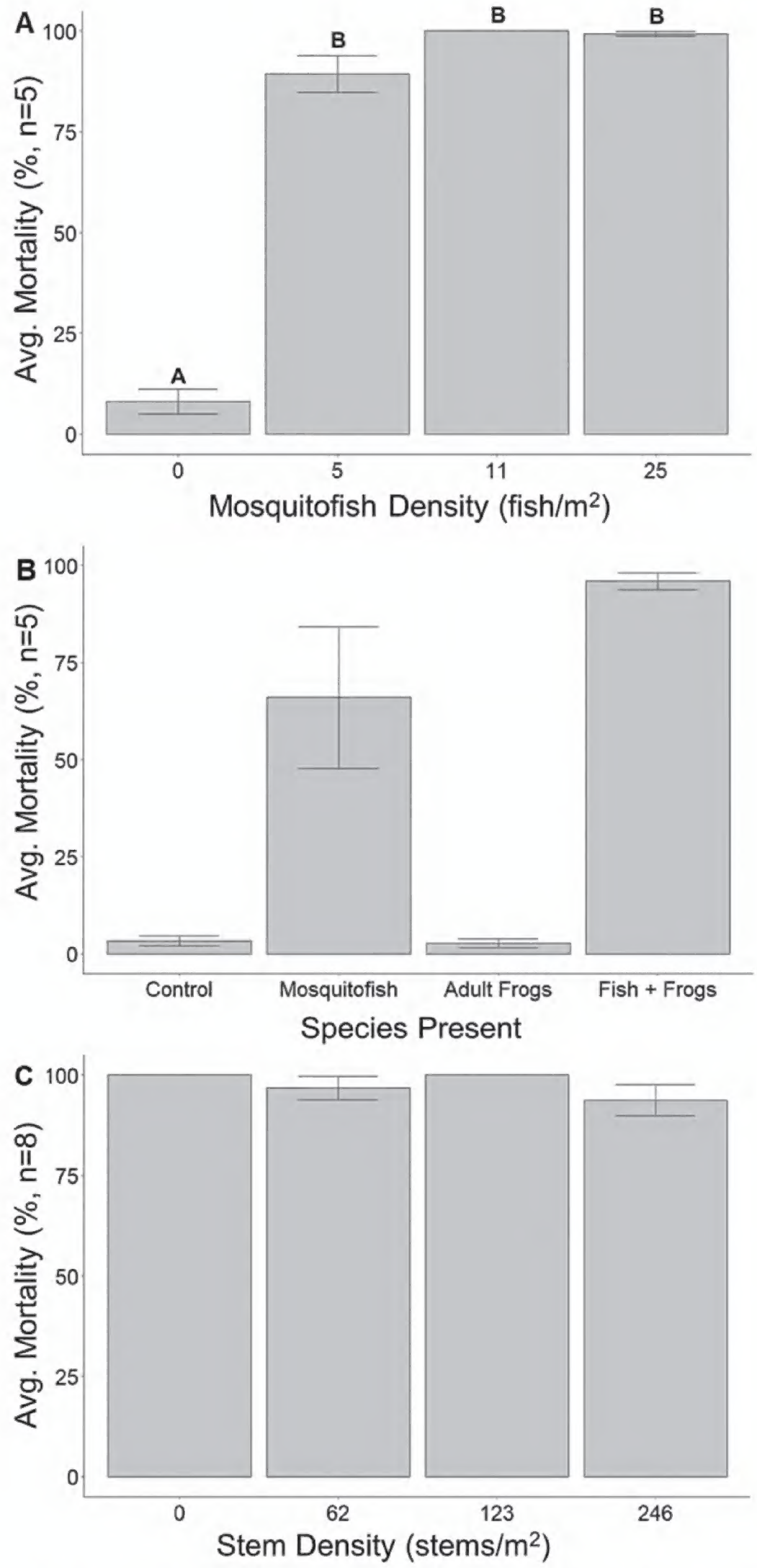

Figure 3. A. Average mortality (%) of tadpoles (± SE) for four mosquitofish stocking densities. Few control tadpoles died and no statistical differences were found between the three mosquitofish stocking densities; **B.** Average percent tadpole mortality between combinations of adult frogs and tadpoles (± SE). The combination of mosquitofish and adult frogs resulted in the highest average mortality of tadpoles, followed by the mosquitofish. Little to no tadpole mortality was observed in the control or adult frog treatments; **C.** Average mortality of tadpoles (± SE) between stem densities in the structural complexity trial. There were no statistical differences in tadpole mortalities between structure densities.

Discussion

Mosquitofish exhibit strong, stage-based biotic resistance to TCFs, with pronounced impacts to egg and larval life stages, but no impacts on adult life stages. Early life stages lack defensive traits, making them highly vulnerable to

mosquitofish predation, fin-nipping and harassment, resulting in near complete mortality. Only when tadpoles begin to metamorphose does survival increase. Once metamorphosis is completed, mosquitofish have no direct effect on juvenile or adult frog survival. The tested levels of mosquitofish density and structural complexity do not alter this relationship, while the presence of adult frogs decreased survival of tadpoles when fish were present. Importantly, if stocked as a part of a targeted biocontrol effort in ephemeral wetlands lacking natural colonisation by fish, mosquitofish predation has the potential to reduce or eliminate TCF recruitment in Florida, starting with the earliest life stages.

Mosquitofish predation was strong in early TCF life stages in our study, although previous studies of fish predation on amphibian eggs have shown mixed results amongst species, with some species experiencing no mosquitofish predation on eggs, but high mortality of newly-hatched larvae (Zeiber et al. 2008; Smith and Smith 2015; Fan et al. 2016). This may be due to species traits, which can modify community interactions, thus modifying biotic resistance, an effect which has been demonstrated across taxa (Smith 2006; Conti et al. 2018). Amphibian eggs can vary in size and thickness of their jelly coating, which may impede predation (Zeiber et al. 2008). Some amphibian eggs are also unpalatable or toxic, as with cane toads (Komak and Crossland 2000). Tropical clawed frog eggs do not exhibit any of these defensive traits, rendering them vulnerable to high levels of mosquitofish predation. Our experiment simulated a scenario in which adult TCFs laid their eggs in a pond occupied by mosquitofish. However, our experiment was conducted in tanks, whereas in a more natural setting, pond characteristics such as turbidity could affect the ability of predators to locate and consume eggs (Zanghi and Ioannou 2025).

All TCF tadpole stages exhibited heavy mortality in the presence of mosquitofish until metamorphic climax was reached. There was a nearly 40% decrease in mortality between the largest pre-metamorphic tadpoles and metamorphosing tadpoles, a result that may be related to tadpole tail damage. Although few attacks were witnessed within the 20-minute observation period of each life stage trial, tail damage was far more prevalent in tanks with mosquitofish, consistent with the findings of Thompson et al. (2012) and Tuckett et al. (2021), which demonstrated similar effects on small-bodied fishes. While tadpole tail injuries are not always fatal, damage can have sub-lethal effects (Blair and Wassersug 2008; Shulse and Semlitsch 2014). Damage to tadpole tails can reduce mobility, impeding their ability to reach the surface to breathe, find food or escape predators (Figiel and Semlitsch 1991). This effect could also explain the increased survival of metamorphs. Metamorphic climax in amphibians is highlighted by growth of functional limbs and absorption of the tail (Gosner 1960), reducing reliance on their tails for locomotion (Sillar et al. 2023). Absorption of a severely damaged tail has been shown to cause delayed metamorphosis in other species, but its effects on the long-term fitness of the adult frog are still unclear (Parichy and Kaplan 1992; da Silva Martins et al. 2022).

Density of the resisting species had minimal effect on tadpole survival, which did not support our hypothesis, although other studies examining biotic resistance found some influence of density on the strength of biotic resistance (Twardochleb et al. 2012; Tuckett et al. 2021). The lowest and highest fish densities used in this experiment caused high mortality of tadpoles and tail damage to the few survivors. This result could suggest two things: 1) tadpole mortality may be the result of both predation and harassment by mosquitofish and 2) even low densities of mosquitofish can have negative impacts on TCF tadpoles. Both have been identified as

important mechanisms of biotic resistance to small-bodied fishes by mosquitofish (Hill et al. 2011; Tuckett et al. 2021). While all densities resulted in the near total elimination of tadpoles after a week in this study, higher mosquitofish densities can influence the overall mortality rate, possibly killing the tadpoles more quickly than would have occurred at a lower density, an effect which may have been masked by the duration of the experiment (Thompson et al. 2012).

The cannibalism experiment resulted in high variability in tadpole mortality. Unlike the structure, stocking density or life stage experiments, the fish alone caused highly variable tadpole mortality. There are limited data to suggest that a species can contribute to its own resistance, although this would be most likely for species with high rates of cannibalism. Cannibalism is well documented in African clawed frogs (McCoid and Fritts 1993; Measey 1998) and was witnessed several times with TCFs in captivity (authors, pers. obs.). Despite this, little to no cannibalism was observed when adults were paired with tadpoles during the course of the experiment. When both adult TCFs and fish were present, tadpole mortality was consistently high and frequently higher than fish alone. These results indicate a multi-predator facilitation effect may be occurring, with predation by either fish or the adult frogs facilitating predation by the other. It seems more likely that mosquitofish are facilitating cannibalism by the adult TCFs. Nearly every surviving TCF tadpole still showed tail damage, which could reduce mobility, facilitating predation by adult TCFs. Facilitation between predators is uncommon, but documented facilitation of cannibalism by another predator is even rarer (Soluk 1993; Eklöv and VanKooten 2001; Schmitz 2007). More study may be required to differentiate the effects of increased predator density from facilitation (Schmitz 2007). There is a growing body of evidence of facilitation in natural systems and this effect can increase the effectiveness of strong predators, potentially enhancing biotic resistance; however, features of the environment and of the predators themselves may result in interference, decreasing biotic resistance (Hill and Floyd 2025).

Unlike some studies (Alexander et al. 2015) and counter to our hypothesis, structural complexity did not reduce predation on tadpoles. The stem density of the habitat had little effect, with poor tadpole survival within the tested stem density ranges. High TCF tadpole mortality in an area with no structure is expected; without refuges, encounters between predators and prey should be higher (Babbitt and Tanner 1997). However, mosquitofish were equally effective within the range of tested stem densities. The stem densities used in this experiment were relatively low compared to other studies (e.g. Thompson et al. (2012)), but mosquitofish are often found in shallow littoral zones and are able to manoeuvre in more densely vegetated areas (Casterlin and Reynolds 1977). While dense vegetation may provide some anuran species refuge from mosquitofish, larger tadpoles that are active in the water column may be more likely to be impacted (Baber and Babbitt 2004). This is comparable to the findings of Shulse and Semlitsch (2014), who found that vegetation cover did not impact tail damage in ranid tadpoles in natural wetlands stocked with mosquitofish. Tropical clawed frog tadpoles are almost always found in the water column as they filter feed and surface to breathe (Wassersug 1996). This may make them easy targets for mosquitofish, attracted to the movement of the tadpoles (Pyke 2005). Therefore, it is likely that mosquitofish could cause comparable tadpole mortality in more structurally complex habitats. At the same time, it is also possible that increasing stem densities can reduce mosquitofish predation efficiency (Baber and Babbitt 2004; Thompson et al. 2012).

From a theoretical perspective, our findings illustrate that biotic resistance acts differentially across the full life cycle of an organism. Likewise, biotic resistance can also act in different stages of invasion; mosquitofish may prevent or reduce invasion of TCFs at the establishment or spread stages (Catford et al. 2009; Blackburn et al. 2011). From an applied perspective, these findings are promising for the control of TCFs in Florida. Biotic resistance provided by mosquitofish outside ephemeral ponds could be augmented into a stage-based biocontrol, targeting TCF eggs and tadpoles, to prevent successful reproduction in new areas and reduce recruitment in ponds where breeding has already occurred. Fish would need to be stocked early in the development of the tadpoles, well before the onset of metamorphosis, to achieve maximum tadpole mortality. Based upon the results of the stem density experiment, mosquitofish should be effective in a variety of wetlands, vegetated or not. The presence of adult frogs in breeding wetlands should not be a hindrance to mosquitofish either and could potentially enhance the biocontrol. Studies are ongoing to determine if these results are generalisable to the invasion in Florida, as well as testing the applicability of this method in the field prior to use as a management technique (Everett 2024). We also caution the use of mosquitofish as a biocontrol outside of its native range or in areas where threatened amphibian species may be breeding, due to the mosquitofish's history of invasion and its possible detrimental impacts on amphibians both in its native and invasive ranges (Pyke 2008).

Conclusions

Biotic resistance will vary across the landscape, varying due to invader identity, species and community interactions and ecosystem features such as habitat structure. This variation will influence its detection and alter its perceived importance. For some species, including multiphasic amphibians, biotic resistance may be strong and stage specific. Thus, the system we describe may be particularly important for testing the biotic resistance hypothesis and further providing information for predictive invasion models or risk assessments for species with complex life histories. The interaction between native mosquitofish and the non-native tropical clawed frog not only provides a model for testing the biotic resistance hypothesis, but from an applied standpoint, can also provide information for augmentative biocontrol, targeting the vulnerable TCF tadpole stages. Future studies need to apply these findings to field settings to assess their applicability.

Acknowledgements

We thank the following UF/IFAS Tropical Aquaculture Laboratory staff and students for assistance with experimental procedures: T. Myles Domohowski, Dylan Niner, Clayton Patmagrian, Amy Wood, Micah Alo, Travis Knorr and the student interns of the Hillsborough Community College aquaculture programme. We also thank Steve Johnson and three anonymous reviewers for their helpful comments on this manuscript. Financial support was provided by the Florida Fish and Wildlife Conservation Commission, the University of Florida (UF) Institute of Food and Agricultural Sciences, Tropical Aquaculture Laboratory (Matthew Di Maggio, Director) and the United States Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA) through Hatch project FLA-FOR-005942. Any opinions, findings, conclusions or recommendations expressed in this report

are those of the authors and do not necessarily reflect the view of USDA or NIFA. The authors followed all relevant institutional guidance for the care and use of vertebrate animals (UF IACUC # 202200000042).

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

Funding

This work was supported by Florida Fish and Wildlife Conservation Commission
National Institute of Food and Agriculture and University of Florida Institute of Food and Agricultural Sciences Tropical Aquaculture Laboratory.

Author contributions

Conceptualization: QMT, KME, JEH. Data curation: KME. Formal analysis: QMT, KME. Funding acquisition: QMT, JEH. Investigation: KME. Methodology: JEH, QMT, KME. Resources: JEH, QMT. Supervision: QMT, JEH. Writing - original draft: KME. Writing - review and editing: QMT, JEH.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Stage based biotic resistance - experimental data

Authors: Katie M. Everett, Jeffrey E. Hill, Quenton M. Tuckett

Data type: xlsx

Explanation note: This data set contains all of the survival data from each experiment listed in the paper, as well as the presence/absence of tail damage of tadpoles from each experiment.

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Link: https://doi.org/10.3897/neobiota.105.165389.suppl1